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The Action of Sodium Hydrosulfite on Selected Flavonoid Compounds

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THE ACTION OF SODIUM HYDROSULFITE ON SELECTED FLAVONOID COMPOUNDS

A thesis submitted by

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NOMENCLATURE

1. Paper Chromatographic Developers:

   (a) BAW, n-butanol-acetic acid-water (4:1:5).

   (b) AHW, acetic acid-concentrated hydrochloric acid-water (30:3:10).

   (c) 6% HOAc, 6% aqueous acetic acid.

   (d) H_2O, distilled water.

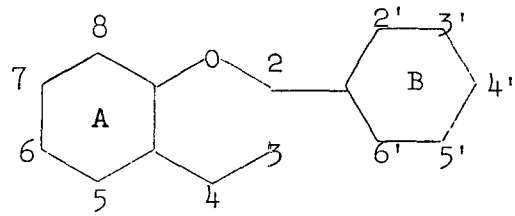
2. Spray Reagents for Paper Chromatograms:

   (a) GG, Fast Red Salt GG, a stabilized diazo salt of p-nitroaniline.

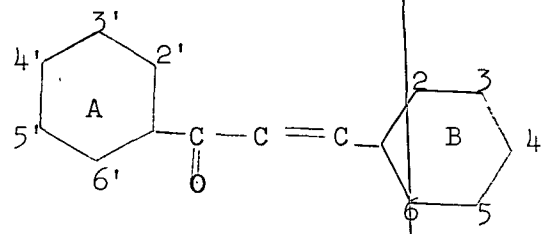
   (b) TSA, 3% solution of p-toluenesulfonic acid in ethanol.

   (c) 2,4-d, 0.05% solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid.

3. Chemical compounds are named in accordance with the nomenclature used by Chemical Abstracts, with the following exceptions. Chemical Abstracts recognizes the name sodium dithionite for the reducing agent sodium hydrosulfite, but because of its acceptance throughout the pulp and paper industry, the latter name is used in this thesis. In flavene compounds, Chemical Abstracts makes no distinction in the location of the double bond. In this thesis the position of the double bond is indicated by the number of the lowest numbered carbon atom to which it is attached. Chemical Abstracts names flavan-3,4-diols and flavan-4-ols as flavans. In this thesis the terms flavan-3,4-diol, and flavan-4-ol are retained, because they have become commonly accepted in the literature, and because the properties of these compounds differ in many respects from those of the flavans. Common names recognized by Chemical Abstracts are used in this thesis. The numbering system used for the flavonoid compounds is illustrated below.



All Flavonoids with a Heterocyclic Ring



Chalcones

As an example of the manner in which this numbering system is employed by Chemical Abstracts, and in this thesis, quercetin is 3,3',4',5,7-pentahydroxyflavone.

INTRODUCTION

Sodium hydrosulfite has been employed commercially for bleaching groundwood pulps since 1932 (1,2). Although the process is limited largely to the west coast where it is used to improve the color of pulps from such species as western hemlock and the western balsam firs, hydrosulfite bleaching ranks second in importance among the groundwood bleaching processes (3), being exceeded only by the peroxide bleaching process. The technology of sodium hydrosulfite bleaching has been well developed, but the mechanism of the bleaching action is not known. In view of the wood species involved and the fact that the pulps are not delignified, it has been suggested that this action is due to the reduction of highly colored wood extractives (1,2,4,5).

One class of wood extractives, the flavonoids, could be of particular importance in the bleaching mechanism, because of the wide distribution of these compounds in wood species used for the manufacture of groundwood pulps, and because the flavonoids include several types of highly colored compounds and the precursors to these and other colored materials. Colored flavonoids originally present in the wood could appear in the pulp, and other colored compounds could be generated from the precursors by chemical action during the grinding process. One kind of chemical transformation which generates colored flavonoids from colorless compounds is mentioned by Shriner (6), and Lewis and Buchanan (7) have discussed other chemical reactions which could occur during grinding. The following discussion deals with the nature of the flavonoid compounds, including properties which could give rise to colored materials during the preparation of a groundwood pulp.

NATURE OF THE FLAVONOID COMPOUNDS

The term flavonoid refers to a group of chemical compounds which possess basic C_6-C_3-C_6 carbon skeletons of the following types (8).

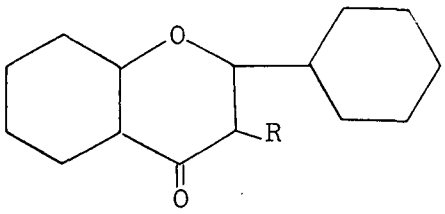
I II

Basic Flavonoid Carbon Skeletons

Substitution of the aromatic rings and variation in the structure of the C_3 portion of the molecule accounts for the wide variety of compounds found among the flavonoids. Concerning aromatic substitution, compounds with many different substituents and substitution patterns are known, but among the naturally occurring compounds the most common substituents are hydroxyl and methoxyl, and the most widely distributed substitution pattern is that in which the A-ring is derived from phloroglucinol and the B-ring from pyrocatechol.

The C_3 structure differentiates the classes of flavonoid compounds. The following survey includes structures and properties of some of the classes of interest in the groundwood bleaching problem.

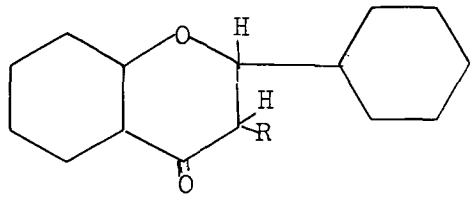
Flavones are yellow, water-insoluble, α,β-unsaturated carbonyl compounds having the following structure.



Flavone Structure; R = H or OH

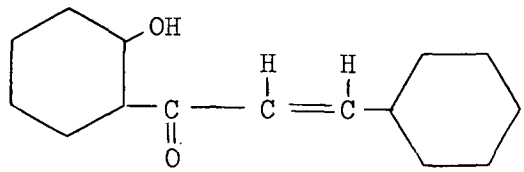
An organic molecule appears colored if its electrons can utilize the energy of radiation in the visible spectrum in undergoing transitions to higher energy states. Since visible radiation is fairly low-energy radiation, the electrons involved in the transitions must be loosely bound; they must behave as though they belong to the entire system rather than a single atom. Such electrons are associated with conjugated systems, and such systems are called chromophores. The greater the number of conjugated bonds, the greater is the chance for visible color to appear. An auxochrome is a group, such as the phenolic hydroxyl group, which can participate in extending the conjugation through ionization or participation in resonance structures (9). In the flavone molecule the yellow color arises from the conjugation of the double bond, the carbonyl group, and the aromatic rings, with phenolic hydroxyl groups participating in extending the conjugation and intensifying the color (10). If the flavone contains a 3-hydroxyl group, a further intensification of the color can arise from the contribution of a possible keto-enol tautomerism (10,11).

Flavanones are water-soluble carbonyl compounds which are colorless because they do not contain the conjugated unsaturation present in the flavones.



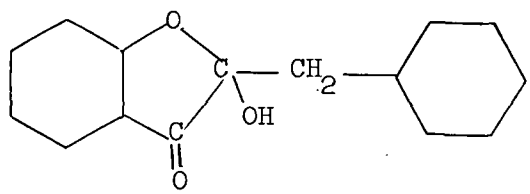
Flavanone Structure; R = H or OH

Flavanones can be oxidized by air to flavones (8). Upon treatment with alkali, flavanones without the 3-hydroxyl group undergo a reversible ring opening to give yellow, water-insoluble 2'-hydroxychalcones (8,12).



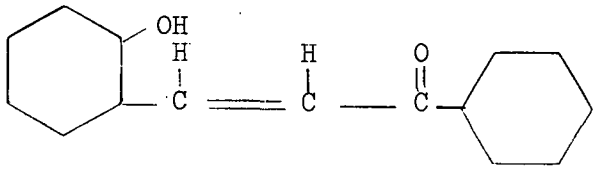
2'-Hydroxychalcone

When the 3-hydroxyl group is present, alkali treatment causes rearrangement of the flavanone ring to give colorless 2-benzyl-2-hydroxycoumaranones (13).



2-Benzyl-2-hydroxycoumaranone

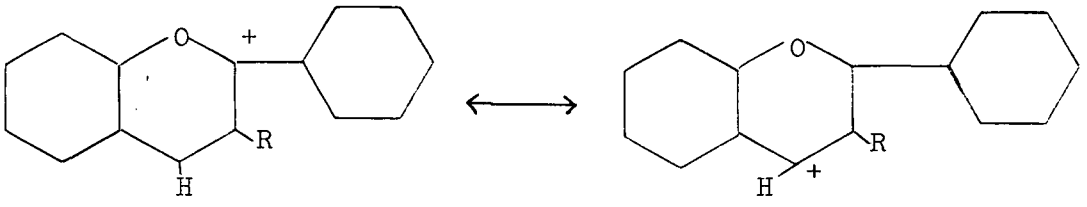
In addition to the 2'-hydroxychalcones which revert to the colorless flavanones upon treatment with acids (8, 12), a less common 2-hydroxychalcone type is known.



2-Hydroxychalcone

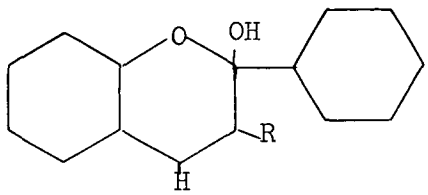
The 2-hydroxychalcones are also yellow, water-insoluble products, but when treated with acids they undergo ring closure with dehydration to give intensely red flavylium salts (14).

Flavylium salts possess resonating carbonium ion structures of the following types (15).

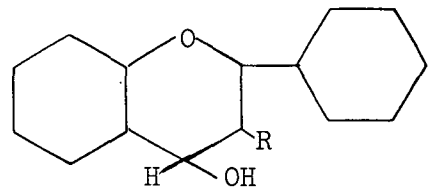


Resonating Flavylium Ion Structures; R = H or OH

3-Hydroxyflavylium salts produce deep red solutions, whereas flavylium salts without the 3-hydroxyl group give orange solutions. This has led to the suggestion of a keto-enol tautomerism in the 3-hydroxy compounds (15). Flavylium salts can be hydrolyzed to colorless products called pseudo-bases. From the resonating flavylium ion structures there should be two possible pseudo-base structures of the hydroxyflavene type (16).



I

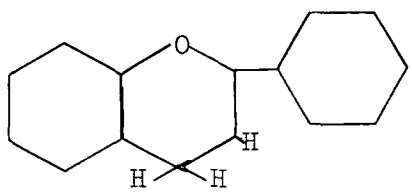


II

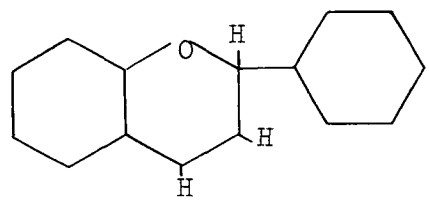
The Two Possible Pseudo-Base Structures; R = H or OH

Structure I is a hemiketal and may undergo ring opening to give a 2-hydroxychalcone (15,17). The action of water on flavylium salts appears to be influenced by structure to some extent. For example, 2-hydroxychalcone is immediately precipitated when a strongly acid solution of flavylium chloride is diluted with water. Hydrolysis of cyanidin chloride, on the other hand, requires 30 minutes of heating to give only the pseudo-base (18).

Flavene compounds are of two types, depending on the location of the double bond in the heterocyclic ring.



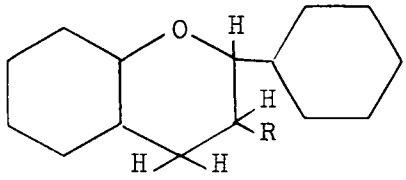
Flavene-2



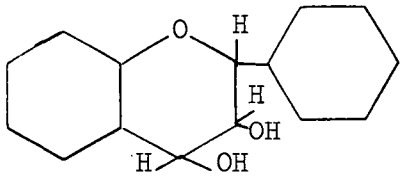
Flavene-3

The pseudo-base types are included in the flavene category. Flavenes are colorless compounds which produce flavylium salts when treated with acid (19-21). Air oxidation of flavenes to uncharacterized yellow products is reported (22). Flavenes with free phenolic hydroxyl groups are reported to be extremely unstable, short-lived compounds (22).

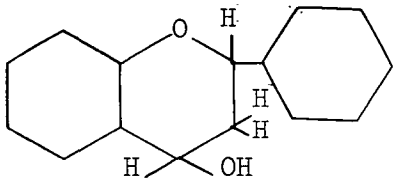
Flavans, flavan-3,4-diols, and flavan-4-ols are colorless, partially water-soluble flavonoid compounds.



Flavan; R = H or OH



Flavan-3,4-diol



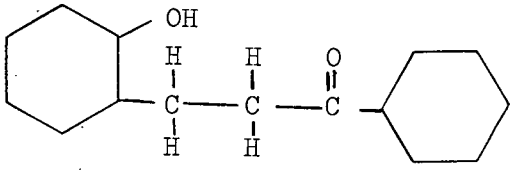
Flavan-4-ol

The flavans readily undergo air oxidation to colored compounds (8), and in the presence of acids they polymerize to complex, brown phlobaphene materials (23), possibly by a mechanism such as that proposed by Brown and coworkers (24,25).

Naturally occurring flavan-3,4-diols belong to a group of compounds called leucoanthocyanidins, which includes, in addition to the flavan-3,4-diols, some polymeric materials of unknown structure. The two types are differentiated on the basis of their chromatographic behavior, the polymeric materials being generally immobile, and the monomeric flavan-3,4-diols being mobile on paper chromatograms (26,27). Both types yield 3-hydroxyflavylium salts and highly complex brown phlobaphenes upon heating with acid (26-31). A suggested mechanism for the flavylium salt formation involves dehydration of the flavan-3,4-diol to a flavene-3 intermediate and oxidation of the intermediate to the flavylium salt (32). The reaction with acid can be used to distinguish the leucoanthocyanidins from other classes of acid-sensitive compounds, since flavenes and pseudo-bases give flavylium salts but no phlobaphene, and flavans give only a phlobaphene (26). Flavan-4-ols give red or orange colors when treated with acid, but

Geissman and Clinton (12) indicate that whereas the colors are due to carbonium ions related to flavylium salts, the colored components are not flavylium salts. Although mineral acids are usually employed, the leucoanthocyanidin reaction has been brought about with weak (2 to 10%) aqueous formic acid solutions (33). The reaction of the leucoanthocyanidins with acid could be an important transformation leading to color in groundwood pulps from such species as western hemlock, since this species gives a pink pulp, and it has recently been found that the wood of this species is very rich in flavan-3,4-diols and polymeric leucoanthocyanidins (34).

β-[2-Hydroxyphenyl]-propiophenone compounds are colorless carbonyl compounds with the following structure.



β-[2-Hydroxyphenyl]-propiophenone

Several authors (21,35,36) have reported reactions of these compounds with acids to give flavylium salts. The mechanism is believed to involve dehydration and oxidation (36).

The above survey of the classes of flavonoid compounds indicates how transformations which could occur during grinding such as air oxidation, the action of acids, and polymerization could give rise to colored flavonoids and polymeric products. The polymeric products are probably

important in the bleaching mechanism, since Curran and co-workers (37) have reported that a phlobaphene material isolated from western hemlock wood was lightened in color by zinc hydrosulfite. However, the structures of these polymeric materials are not yet known; so the chemical changes involved cannot be determined. From the nature and structure of the colored flavonoid compounds, on the other hand, it is not difficult to see that one way to render these molecules colorless would be to destroy the conjugation responsible for the color by reduction.

REDUCTION OF COLORED FLAVONOID COMPOUNDS

FLAVONES

The behavior of the flavone molecule upon reduction seems to depend largely on the reducing agent employed and the conditions used, with the result that a variety of products have been recorded. In catalytic hydrogenation, for example, where reduction is known to occur in a stepwise manner* (38), it is possible to obtain products ranging from flavanones (39,40) to flavan-3,4-diols and flavan-4-ols (41,42) by choosing the proper reaction conditions.

Some reducing agents act only upon the carbonyl group, but the type of product seems to depend on the reducing agent employed. For example,

*When a molecule contains several reducible groups the general reduction sequence in catalytic hydrogenation is as follows: The olefinic double bond and nitro group are most easily reduced, followed closely by the carbonyl group, the cyano group, and aliphatic hydroxyl, the carbocyclic systems, the heterocyclic systems, and finally the very resistant carboxyl group (38).

magnesium and hydrochloric acid converts flavones to more intensely colored flavylium salts (8,15,43). Lithium aluminum hydride, on the other hand, gives 4-hydroxyflavene-2 compounds (19).

The ease of reduction of the carbonyl group is influenced by substitution. Geissman and co-workers (44) found, upon reducing flavones at a dropping mercury electrode, that if hydroxyl groups were present in the 3,4',5, or 7-positions, the carbonyl group was difficult to reduce. The 3 and 5-hydroxyls can form hydrogen bonds with the carbonyl group, making the carbonyl oxygen more negative and less easily reduced. Hydroxyls in the 4',5, and 7-positions can participate in resonance structures involving the carbonyl group, again lending negative character to the carbonyl oxygen. The products of these electrolytic reductions were 4-hydroxyflavene-2 compounds.

Reducing agents which apparently attack only the double bond in the flavones are known. Zinc and acid reduced 3-hydroxyflavones to the corresponding 3-hydroxyflavanones (40), as did sodium hydrosulfite in alkali (40,45-47). The hydrosulfite reductions were carried out on naturally occurring flavones in a strongly alkaline medium where the flavones were soluble. The highest yield of flavanone was about 35%.

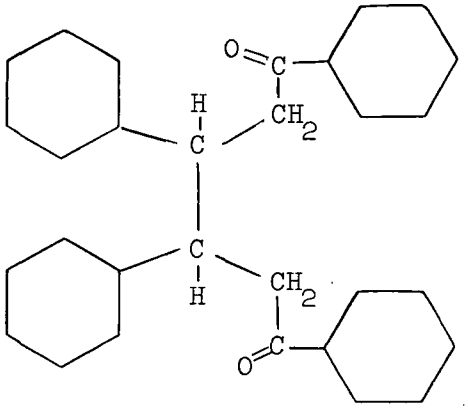
CHALCONES

The behavior of the chalcones upon reduction is similar in many respects to that of the flavones. For example, catalytic hydrogenation can give different products depending on the choice of conditions. Catalytic hydrogenation of chalcones under mild conditions is the common method for

preparing β-phenylpropiophenone compounds (35,48), but if the reaction is allowed to continue or if more severe conditions are used, reduction to propanols results (48). As in the flavones, the ease of reduction of the chalcone carbonyl group is influenced by substitution. Hydroxyl groups on the aromatic rings in such positions that they can participate in resonance or hydrogen bonding with the carbonyl oxygen make reduction of the carbonyl group more difficult (49). Again, as in the flavones, certain reducing agents, notably sodium borohydride (50) and lithium aluminum hydride (51), attack only the carbonyl group to give unsaturated alcohols.

Polarographic studies (49) indicate that electrolytic reduction of chalcones proceeds by a free radical mechanism in which the double bond is first saturated to give β-phenylpropiophenones. The carbonyl group is then reduced to give either a propanol or a pinacol.

Zinc and acetic acid has been recommended (52) for reducing double bonds in α,β-unsaturated ketones without attacking carbonyl groups. It would thus be expected that zinc and acetic acid should produce β-phenylpropiophenones from chalcones. Instead, coupling apparently takes place to give 1,3,4,6-tetraphenylhexane-1,6-diones (53,54).



1,3,4,6-Tetraphenylhexane-1,6-dione

The action of sodium hydrosulfite on the chalcones has not been investigated.

FLAVYLIUM SALTS

Catalytic hydrogenation of flavylium salts gives colorless flavans (22,55) by a two-step reaction. In the first step the flavylium salt rapidly takes up one mole of hydrogen to give the corresponding flavene-2, and in the absence of water the flavene-2 slowly takes up a second mole of hydrogen to give the flavan (55). Freudenberg and Harder (55) found in one instance that when water was present, hydrogenation of the flavene-2 did not occur. Instead, the flavene-2 added water to give a β-[2-hydroxyphenyl]-propiophenone derivative.

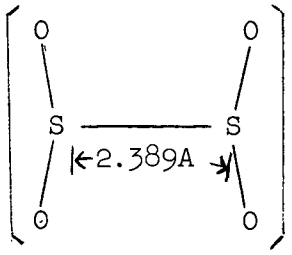
Reduction of flavylium salts with lithium aluminum hydride gives flavene-3 compounds (22,56). These products, like the flavene-2 compounds, can be catalytically hydrogenated to flavans (56,57).

The decolorization of flavylium salt solutions by sodium hydrosulfite, by zinc and acetic acid, and by sodium bisulfite has been reported by several workers with the implication that all of these reducing agents produced the same chemical changes (58-61). In most of the instances, however, nothing was done to determine what changes had taken place. Kuhn and Winterstein (59) suggested that zinc and acetic acid reacted with cyanidin chloride to give a 3-hydroxyflavene-3, but Freudenberg and Steinbrunn (62) were unable to catalytically hydrogenate the product to the 3-hydroxyflavan. It is, therefore, unlikely that a flavene-3 was present. At the present time it still remains to be established that the three

reducing agents have the same chemical action, but more importantly, the mechanism of the decolorizing action of sodium hydrosulfite has not been determined.

CHEMISTRY OF SODIUM HYDROSULFITE

The planar hydrosulfite anion is regarded as a pair of SO_2^- units linked by a weak sulfur-sulfur single bond (63,64).



The Hydrosulfite Anion

Polarographic studies on sodium hydrosulfite solutions (65) and studies on the exchange of sulfur between hydrosulfite ion and sulfur dioxide (66) indicate that the SO_2^- species exists in equilibrium with the hydrosulfite ion in sodium hydrosulfite solutions. From a structural standpoint, therefore, the reducing action of sodium hydrosulfite might be regarded as the reaction of the SO_2^- unit to give sulfur dioxide and an electron (63). The standard reduction potentials and the corresponding half-cell reactions for sodium hydrosulfite in acid and alkaline solution are given by Latimer (67) as follows:

in acid solution,

$$HS_2O_4^- + 2H_2O = 2H_2SO_3 + H^+ + 2e^- \qquad E^\circ = 0.08 \text{ volt;} \quad (1)$$

in alkaline solution,

$$S_2O_4^= + 4OH^- = 2SO_3^= + 2H_2O + 2e^- \qquad E_B^\circ = 1.12 \text{ volts.} \quad (2)$$

Sodium hydrosulfite is very unstable in solution. The most important decomposition reaction forms thiosulfate and bisulfite (67,68) as illustrated in Reaction (3).

$$2S_2O_4^{=} + H_2O \longrightarrow S_2O_3^{=} + 2HSO_3^{-} \qquad (3)$$

According to Barton (68), at 60°C. a 2% solution of sodium hydrosulfite will be about 50% decomposed by Reaction (3) in one hour and about 80% decomposed after three hours. The decomposition rate is increased by increasing the temperature or lowering the pH. The reaction is inhibited by alkali. Small amounts of sulfides and free sulfur are also found in decomposed hydrosulfite solutions, but the mechanism of their formation is not known. When exposed to air sodium hydrosulfite is oxidized to sodium bisulfate and sodium bisulfite (68).

When used as a reducing agent in organic reactions, sodium hydrosulfite is employed most often in an alkaline medium because of its reducing power and its stability under these conditions. Sodium hydrosulfite has been used to reduce olefinic double bonds in unsaturated ketones without reducing the carbonyl groups (52,69), but this does not mean that sodium hydrosulfite has a specific reducing action. Pearl has found, for example, that one of the two carbonyl groups is vanillil and in syringil is reduced to an alcohol by sodium hydrosulfite (70,71).

When used as a bleaching agent for groundwood pulps, sodium hydrosulfite is employed at a neutral pH. Alkaline conditions cannot be used, because alkali has a detrimental effect on the pulp (68). A typical groundwood bleaching operation is carried out at a pulp consistency of about 3%

with 0.2 to 0.5% of sodium hydrosulfite, based on the airdry weight of the pulp, being applied as a 1% aqueous solution. The pH is between 6.0 and 7.5, the temperature is about 60°C., and the retention time ranges from 15 minutes to one hour at this temperature (68,72,73).

STATEMENT OF THE PROBLEM

The mechanism of the bleaching action of sodium hydrosulfite on groundwood pulps is not known. The determination of this mechanism can be set as an ultimate goal to be achieved, and to which the present research is directed. It is often hypothesized that the sodium hydrosulfite bleaching action involves the reduction of highly colored extractives present in the pulps because they were present in the wood or because they could be generated from colorless compounds by chemical action during the grinding operation. Accepting this hypothesis, the problem becomes one of determining how sodium hydrosulfite reacts with such compounds under bleaching conditions. Among the classes of compounds which could contribute significantly to the color of groundwood pulps are the colored flavonoid compounds.

Previous investigations of the reduction of flavonoid compounds with sodium hydrosulfite are not directly applicable to the problem of determining the mechanism of groundwood bleaching. Flavone reductions were all carried out under strongly alkaline conditions. The chemical action involved in the decolorization of flavylium salts was not investigated. Reactions of chalcones with sodium hydrosulfite have not been reported. The present work was undertaken to obtain information about the behavior of the flavonoids which could be applied to the groundwood bleaching problem. Specifically, the objective of this research was to determine how flavonoids primarily of the colored flavone, chalcone, and flavylium salt types behave with sodium hydrosulfite under conditions approximating groundwood bleaching conditions.

GENERAL EXPERIMENTAL METHODS

PREPARATION AND PROCUREMENT OF STARTING COMPOUNDS

QUERCETIN AND DIHYDROQUERCETIN

Quercetin (3,3',4',5,7-pentahydroxyflavone) and dihydroquercetin (3,3',4',5,7-pentahydroxyflavanone) were obtained from Krischell Laboratories, Portland, Oregon. The quercetin melted at 312 to 314°C., and the dihydroquercetin melted at 232 to 235°C. Both materials were chromatographically homogeneous when first obtained. Upon prolonged exposure to air, some oxidation of the dihydroquercetin to quercetin occurred.

CHALCONES

All chalcones employed in this study were synthesized by condensing the appropriate aromatic aldehyde with the appropriate acetophenone in the presence of alkali. The following compounds were prepared.

2-Hydroxychalcone

A mixture of 7 grams of salicylaldehyde and 7 grams of acetophenone was allowed to stand with 25 ml. of 60% potassium hydroxide solution at 0°C. for two days. The orange crystals which had separated were recovered and dissolved in water. Upon acidifying this solution, yellow crystals separated. The crystals were recovered by filtration in a yield of 4 grams. After two recrystallizations from 95% ethanol, the melting point was 151 to 153°C., and was unchanged by a third recrystallization. Bablich and Kostanecki (74), who originally prepared this substance by essentially the same procedure, recorded a melting point of 153 to 155°C.

2'-Hydroxychalcone

The procedure for synthesizing 2'-hydroxychalcone was a modification of the procedure followed in the original preparation (75). To 3 grams of o-hydroxyacetophenone and 3 grams of benzaldehyde were added 10 ml. of 60% aqueous potassium hydroxide solution. After five days at room temperature the mixture was diluted with ice water, acidified with 5N hydrochloric acid, and extracted with ether. The extract was washed with dilute sodium bisulfite solution and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The yellow solid (1.7 gram yield) was recrystallized once from 50% ethanol, after which it melted at 88 to 89°C. The melting point recorded for 2'-hydroxychalcone is 88 to 89°C. (75).

2',4,4',6'-Tetrahydroxychalcone

The procedure used for 2'-hydroxychalcone was employed to prepare 2',4,4',6'-tetrahydroxychalcone from 2.5 grams of phloroacetophenone and 2.5 grams of p-hydroxybenzaldehyde. The ether extract yielded 2 grams of yellow crystals which were recrystallized from 50% ethanol. The product darkened to a brown color at 155°C. and melted slowly at 244 to 248°C. Physical data for this chalcone are not recorded in the literature, but the corresponding colorless flavanone naringenin melts at 247 to 248°C. (76). It appears, from discussions such as that of Zemplén and co-workers (76), that the chalcone is very readily converted to the flavanone, and that the term naringenin may be used to name both forms.

FLAVYLIUM SALTS

All flavylium salts were prepared by condensing the proper aromatic aldehyde with the proper acetophenone by means of hydrogen chloride. The following compounds were prepared.

Cyanidin Chloride (3,3',4',5,7-Pentahydroxyflavylium Chloride)

The method of Robertson and Robinson (77) was used to prepare cyanidin chloride. The details were as follows.

α,3,4-Triacetoxyacetophenone

A mixture of 10 grams of α-chloro-3,4-dihydroxyacetophenone, 10 grams of potassium acetate, and 40 ml. of acetic anhydride was heated on a steam bath for three hours. Upon cooling and pouring the mixture into 400 ml. of water, a brown sirup which soon solidified was obtained. The solid was recovered and recrystallized from carbon tetrachloride and from methanol to give 9 grams of light tan crystals melting at 95°C.

o-Benzoylphloroglucinaldehyde

A solution of 1.7 grams of potassium hydroxide in 150 ml. of water was cooled to -5°C., and 5.2 grams of phloroglucinaldehyde were added. Three 2.35-gram portions of benzoyl chloride were added with vigorous agitation, the agitation being continued until the benzoyl chloride odor has disappeared. The mixture was stirred with an excess of 8% aqueous sodium bicarbonate solution for 30 minutes. The resulting orange solid was filtered off, washed with water, and air dried. The solid was dissolved in 100 ml. of hot methanol and the solution was poured into 400 ml.

of water to get very fine, pale orange needles which were recovered and dried in a desiccator over phosphorus pentoxide. The yield was 5 grams, melting point 198 to 200°C.

5-Benzoylcyanidin Chloride

In a mixture of 50 ml. of ethyl acetate and 20 ml. of absolute ethanol were dissolved 2.5 grams of o-benzoylphloroglucinaldehyde and 3.5 grams of α,3,4-triacetoxyacetophenone. The solution was cooled to 0°C., saturated with hydrogen chloride, and placed in a refrigerator for three days. The crude product was recovered and recrystallized by dissolving in a boiling mixture of ethanol and 0.5% hydrochloric acid (1:1 by volume), adding hot 10% hydrochloric acid, and distilling off the alcohol until crystals appeared. The product separated upon cooling, and 5 grams were recovered and air dried.

Cyanidin Chloride

The above product was debenzoylated by dissolving in 65 ml. of cold 8% sodium hydroxide (in a solvent consisting of three volumes of ethanol and two volumes of water) and allowing the resulting blue solution to stand under nitrogen for three hours with occasional swirling. The solution gradually became dull orange-brown. Concentrated hydrochloric acid (100 ml.) was added, and the mixture was heated at 60°C. until the color was a deep red-violet. After standing overnight at 0°C., the solid was filtered off, washed with 5% hydrochloric acid and ether, and air dried. The product was purified by dissolving it in ethanol, adding 10% hydrochloric acid, and distilling off alcohol until solid appeared. The product

separated upon cooling as deep red crystals which were recovered and air dried. The yield was 3 grams.

Pseudo-Base of Cyanidin Chloride

A portion of the cyanidin chloride was hydrolyzed to the pseudo-base by the procedure of Willstätter and Nolan (18). In 830 ml. of 95% ethanol and 1640 ml. of water was dissolved 0.5 gram of cyanidin chloride. The deep red-violet solution was heated for 30 minutes at 80°C., during which time the color gradually lightened to a faint pinkish orange. The alcohol was then rapidly distilled off. Although the authors record the isolation of the crystalline pseudo-base, the freshly prepared solution was used in the present work without isolating the product. Chromatograms of the freshly prepared solution showed a single component with the following behavior.

R_f Values			Color Reactions					
			GG			TSA		2,4-d
BAW	6% HOAc	H_2O	vis.	UV		vis.	UV	
0.70	0.28	0.22	C	O	YBr	P	RO	O

Legend: C = colorless, O = orange, R = red, Br = brown, P = pink, Y = yellow

Fisetinidin Chloride (3,3',4',7-Tetrahydroxyflavylium Chloride)

The procedure of León and Robinson (78), as used by Freudenberg and Maitland (23), was employed for this preparation. A solution of 4.2 grams of α,3,4-triacetoxyacetophenone and 2.1 grams of β-resorcylaldehyde in 63 ml. of formic acid was saturated with hydrogen chloride at 0°C. After standing for 30 hours at room temperature the deep red solution was poured into 875 ml. of anhydrous ether, and the red solid which separated was

filtered off. The solid was heated with 100 ml. of 95% ethanol and 18 ml. of concentrated hydrochloric acid for 20 minutes. Upon cooling, the product crystallized. The yield was 2.75 grams after recrystallizing from 0.5% hydrochloric acid.

3,3',4'-Trihydroxyflavylium Chloride

The same general procedure used for preparing fisetinidin chloride was employed for 3,3',4'-trihydroxyflavylium chloride. A solution of 3 grams of α,3,4-triacetoxyacetophenone and 15 ml. of salicylaldehyde in 25 ml. of ethyl acetate was satúrated at 0°C. with hydrogen chloride. After standing overnight at room temperature the dark red solution was poured into 500 ml. of anhydrous ether. The red solid which separated was filtered off and air dried. The yield was 2 grams.

Luteolinidin Chloride (3',4',5,7-Tetrahydroxyflavylium Chloride)

The procedure of León and Robinson (78) was followed in synthesizing this compound. The yield was very low, however, and it was difficult to obtain a chromatographically homogeneous product.

3,4-Diacetoxyacetophenone

To a solution of 30 grams of α-chloro-3,4-dihydroxyacetophenone in 2 liters of 95% ethanol were added 100 grams of zinc dust, 50 ml. of glacial acetic acid, and 350 ml. of water. This mixture was kept at 40°C. for an hour. Another 50 ml. of acetic acid and 350 ml. of water were added, and the 40°C. temperature was maintained for two hours. The zinc was filtered off, the filtrate was concentrated to 100 ml., and 500 ml. of

water were added. The solution was extracted with three 500-ml. portions of ether, and the extract was evaporated to dryness. The residue was recrystallized from 2 liters of chloroform with the aid of activated charcoal to give 11.6 grams of colorless 3,4-dihydroxyacetophenone melting at 119 to 121°C. Freudenberg and Maitland (23) record a melting point of 122°C. This product (10 grams) was acetylated with 10 grams of sodium acetate and 50 ml. of acetic anhydride by heating the mixture on a steam bath for two hours and then pouring into 300 ml. of ice water. Upon standing, colorless crystals settled out. The yield was 9.8 grams, melting point 86 to 88°C. Freudenberg and Maitland (23) record 88 to 90°C. for the acetyl derivative.

5-Benzoylluteolinidin Chloride

A solution of 2 grams of o-benzoylphloroglucinaldehyde and 1.37 grams of 3,4-diacetoxyacetophenone in 50 ml. of ethyl acetate was saturated at 0°C. with hydrogen chloride. A red solid was filtered off, and the filtrate was poured into much anhydrous ether, whereupon additional product separated. The total yield was only 0.6 gram. The substance decomposed at 179 to 182°C. without melting.

Luteolinidin Chloride

The above product was dissolved in cold 8% sodium hydroxide solution (the solvent was 3:2 ethanol-water by volume) and was kept under nitrogen for three hours, during which the color changed from deep blue to orange-brown. Concentrated hydrochloric acid (12 ml.) was added, and the mixture was heated at 60°C. until the color of the solution was deep red-orange.

The product separated upon standing in the refrigerator as red-orange crystals. The yield was 0.3 gram.

Butinidin Chloride (3',4',7-Trihydroxyflavylium Chloride)

The procedure of Freudenberg and Maitland (23) was followed for preparing this compound. A mixture of 3.76 grams of β-resorcylaldehyde, 6.6 grams of 3,4-diacetoxyacetophenone, and 73 ml. of ethyl acetate was saturated at 0°C. with hydrogen chloride. After 48 hours at room temperature the ethyl acetate was decanted and the residue was triturated with 170 ml. of cold methanol. The solid material was filtered off, and a few drops of 5N hydrochloric acid was added to the filtrate, which was then heated with 200 ml. of 10% hydrochloric acid. Upon standing overnight the product crystallized from the solution. A yield of 2 grams of bright red crystals was obtained.

3'-Methoxy-4'-dihydroxyflavylium Chloride

The preparation of this compound from 3'-methoxy-2,4'-dihydroxychalcone has been described by Sen (79), but a direct condensation with hydrogen chloride was employed here. A mixture of 8 grams of salicylaldehyde, 10.8 grams of acetovanillone, and 20 ml. of glacial acetic acid was saturated at 0°C. with hydrogen chloride. Upon standing overnight at room temperature, part of the product crystallized and was filtered off. The filtrate was poured into anhydrous ether to separate more of the product. The combined products were recrystallized from 70% ethanol which had been saturated with hydrogen chloride. The yield was 5 grams.

Flavylium Chloride

Flavylium chloride is unstable and exists only in strongly acid solution (80). For a solution of flavylium chloride, 2-hydroxychalcone was dissolved in glacial acetic acid, and this solution was boiled for a few minutes with concentrated hydrochloric acid until the yellow chalcone color had become deep red-orange and did not change further. When such a solution was diluted with water, the chalcone was reprecipitated.

SODIUM HYDROSULFITE REDUCTIONS

All flavonoid reductions with sodium hydrosulfite were performed in an aqueous medium containing essentially 1% by weight of sodium hydrosulfite and 0.05% by weight of the flavonoid compound. Reactions were carried out at pH 7 and 60°C. in an atmosphere of nitrogen for a period of one hour.

The reaction vessel was a 3000-ml., three-neck, round-bottom flask. A rubber stopper in the large center neck accommodated an electric stirrer, a buret, and a nitrogen inlet tube which extended to within an inch of the surface of the reaction mixture. The hole through which the stirrer passed was enlarged to provide an outlet for the nitrogen. A thermometer was inserted through one of the smaller side necks, and a 25-ml. pipet was inserted through the other side neck.

Reaction mixtures were prepared by heating 2000 ml. of distilled water at 60°C. while passing nitrogen from a cylinder through the flask. The flavonoid compound (1.0 gram) and sodium hydrosulfite (20.0 grams)

were added, and the pH was rapidly adjusted to seven by adding 10% aqueous sodium carbonate solution from the buret. Samples of the mixture were removed with the pipet for pH measurement on a Beckman model H-2 pH-meter. After this initial pH adjustment, the pH was monitored at 15-minute intervals during the reaction period.

A few preliminary experiments were carried out at one-tenth the scale of the main experiments described above, using a 500-ml. three-neck flask as the reaction vessel.

At the end of the reaction period the reduction mixtures were cooled rapidly under cold tap water. Undissolved material, if any, was filtered off, and the solutions were extracted with ethyl acetate. In the few preliminary experiments, extraction was carried out in a separatory funnel, but in the main experiments a gas-activated continuous liquid-liquid extractor of the type described by Pearl (81) was employed, using nitrogen to provide mixing and an inert atmosphere.

PAPER CHROMATOGRAPHY

Paper chromatography was used to detect reaction products in the ethyl acetate extracts, to monitor separation and isolation techniques, and to apply certain qualitative functional group tests to the products. Chromatograms were developed by the descending method and then removed from the tanks and air dried. The dried chromatograms were examined under ordinary and ultraviolet light and then sprayed with various spray reagents.

Solvent systems used for developing the Whatman No. 1 paper chromatograms were as follows:

(a) the upper phase of n-butanol-acetic acid-water (4:1:5)(BAW)(82);

(b) 6% aqueous acetic acid;

(c) distilled water (82);

(d) acetic acid-concentrated hydrochloric acid-water (30:3:10)(AHW)(83).

The spray reagents used on the paper chromatograms included:

(a) Fast Red Salt GG (a stabilized diazo salt of p-nitroaniline)* (84). A 0.05% aqueous solution was prepared just prior to use. The sprayed chromatograms were air dried and then sprayed with a 10% aqueous sodium carbonate solution. All phenolic materials encountered in this work gave color reactions with this spray reagent.

(b) p-Toluenesulfonic acid in ethanol (27). Crystalline p-toluenesulfonic acid was dissolved in absolute ethanol to give a 3% solution. The sprayed chromatograms were air dried and then placed in an oven at 105°C. to develop the color. The chromatograms were then examined under ordinary and ultraviolet light. This reagent gives characteristic pink or orange colors with leucoanthocyanidins due to generation of the flavylium salts.

(c) 2,4-Dinitrophenylhydrazine in 2N hydrochloric acid. 2,4-Dinitrophenylhydrazine was dissolved in 2N hydrochloric acid to give a 0.05% solution. Carbonyl compounds developed bright yellow to orange spots on a light yellow background. However, compounds which could be oxidized by the reagent were also found to give color reactions.

(d) 2% Ferric chloride in water. This reagent was used occasionally to detect simple phenolic materials.

* A product of Antara Chemical Division, General Aniline and Film Corporation.

(e) n-Propanol 0.3N in hydrochloric acid (30). This spray was employed in the same manner as p-toluenesulfonic acid for detecting leucoanthocyanidins and other compounds giving color reactions with strong acids.

(f) Hydrochloric acid vapors. These vapors were sometimes used to detect leucoanthocyanidins and other compounds giving color reactions with strong acids. Chromatograms were placed in a desiccator over concentrated hydrochloric acid until the color developed.

SOLVENTS AND REAGENTS

All solvents and reagents used were reagent grade, unless otherwise noted in the text.

DETERMINATION OF INFRARED SPECTRA

All infrared spectra determinations were made with a Perkin-Elmer Model 21 Recording Infrared Spectrophotometer by Mr. Lowell Sell of the Analytical Department of The Institute of Paper Chemistry. Samples for spectra determination were prepared as potassium bromide pellets.

MICROANALYTICAL DETERMINATIONS

Microanalytical determinations of carbon, hydrogen, oxygen, and acetyl group contents of reaction products and derivatives were made by the Huffman Microanalytical Laboratories, Wheatridge, Colorado.

EXPERIMENTAL RESULTS AND DISCUSSION

REDUCTION OF QUERCETIN AND DIHYDROQUERCETIN

Quercetin did not dissolve in the sodium hydrosulfite solution under the conditions employed in this study, and it was recovered quantitatively by filtering the mixture at the end of the reaction period. The recovered solid was compared with authentic quercetin by melting point and mixed melting point determinations and by paper chromatography, and the two materials corresponded in these respects. Paper chromatograms of an ethyl acetate extract of the filtered hydrosulfite solution were blank. The same results were obtained when the experiment was repeated.

To determine whether or not the absence of reaction in the neutral medium was due to the insolubility of the quercetin, a slurry of 0.1 gram of quercetin and 0.025 gram of magnesium carbonate in 200 ml. of water was heated at 60°C. for 15 minutes to form a weak, water-soluble quercetin-magnesium complex described by Watkin (85). The resulting orange solution was treated with sodium hydrosulfite at pH 7 under the small-scale reaction conditions. A color change from orange to light yellow occurred almost immediately, but the reaction was allowed to proceed for the full hour. Paper chromatograms of the ethyl acetate extract showed two spots, one corresponding to unreacted quercetin and the other corresponding to authentic dihydroquercetin.

Dihydroquercetin was treated with sodium hydrosulfite under the small-scale reaction conditions. Although it was readily soluble in the

reaction medium, dihydroquercetin apparently did not undergo reduction. Chromatograms of the ethyl acetate extract showed spots only for unreacted dihydroquercetin.

Quercetin was insoluble in the reduction medium and did not react with sodium hydrosulfite at pH 7. The evidence indicated that the insolubility was the primary reason for the absence of reaction, since reduction to dihydroquercetin occurred when the quercetin was rendered soluble. The solubility behavior of other flavones is the same as that of quercetin; so other flavones should be expected to behave toward sodium hydrosulfite in the same way as quercetin. For this reason, no further studies of the reduction of flavones were made. Under alkaline conditions and when made soluble at pH 7, the flavones are reduced to flavanones. The flavanone dihydroquercetin did not react further with sodium hydrosulfite at pH 7.

REDUCTION OF CHALCONES

Like quercetin, 2',4,4',6'-tetrahydroxychalcone was initially insoluble in the reduction medium. However, the chalcone slowly dissolved to give a colorless solution after one hour. The ethyl acetate extract from this colorless solution was bright yellow, and chromatograms of the extract showed spots only for the unreacted chalcone. Recovery of the chalcone by evaporating the extract to dryness was quantitative.

Reduction of 2'-hydroxychalcone proceeded in the manner described above. The compound was initially insoluble, but gradually dissolved to give a colorless solution. Again the ethyl acetate extract was bright

yellow, chromatograms of the extract showed only unchanged chalcone, and recovery of 2'-hydroxychalcone from the extract was quantitative.

2-Hydroxychalcone appeared to behave initially in the same way as the above chalcones. Once more a colorless solution was obtained as the chalcone gradually dissolved in the reduction medium, and again extraction gave a bright yellow extract. In this case, however, chromatograms of the extract showed two spots. A very intense spot for the chalcone was present, as well as a faint spot for a reduction product. The chromatographic behavior of this reaction product, designated Compound K, appears in Table I.

TABLE I

CHROMATOGRAPHIC BEHAVIOR OF COMPOUND K

R_f Values				Color Reactions[c]					
BAW	6% HOAc	H_2O	AHW	vis.	UV	GG	TSA vis.	TSA UV	2,4-d
0 59	0.2-0.4[a]	0.1-0.3[a]	0.86[b]	C	C	Y	Y	Y	bY

[a] In 6% HOAc and H_2O, the component gave streaks with the indicated approximate R_f ranges.

[b] In AHW a yellowish orange spot was found at the indicated R_f value. This spot corresponded to an authentic sample of flavylium chloride.

[c] Legend: b = bright; C = colorless; Y = yellow.

Compound K was recovered by evaporating the ethyl acetate extract to dryness, taking up the residue in water, filtering off the unreacted chalcone, concentrating the aqueous filtrate to a volume of 1 ml., and allowing this solution to stand overnight in a refrigerator. Six milligrams of silvery gray plates were thus obtained. Chromatograms of the aqueous filtrate no longer showed a spot for Compound K. The crystals

melted at 87 to 88°C. Owing to the small amount of product, purification was not attempted. The spectrum of Compound K is shown in Fig. 1. This spectrum shows a band at 5.96 μ which indicates carbonyl, a band at 3.5 μ indicating $-CH_2-$, a band at 3.0 μ indicating hydroxyl, and various aromatic and phenolic bands. Bands characteristic of olefinic unsaturation are not present. The infrared spectrum and the conversion to flavylium chloride in the acidic AHW solvent suggest that Compound K is β-[2-hydroxyphenyl]-propiophenone, which melts at 91 to 92°C. when pure (86). Few details could be found for the preparation of this propiophenone in the literature, and attempts to prepare it by catalytic hydrogenation of 2-hydroxychalcone at room temperature with a 5% palladium-charcoal catalyst and with Raney nickel gave products which did not contain a carbonyl group.

Chalcones are usually considered to be water-insoluble compounds, but the chalcones studied in this work gradually dissolved in the sodium hydrosulfite medium to give colorless solutions. However, evidence of reduction was found only in the case of 2-hydroxychalcone, and even in this case reduction appeared to be slight. The absence of color in the chalcone solutions does not appear to be due to isomerization to colorless flavanones (12) or to the formation of stable sulfonates by the action of bisulfite (87). These products are stable and should have been readily detected if present. Also, the chalcones were recovered quantitatively in all instances except that of 2-hydroxychalcone. Air oxidation of a reduction product back to the chalcone during the extraction is not likely, because the extraction was carried out under nitrogen, and the product from 2-hydroxychalcone appeared to be stable in air.

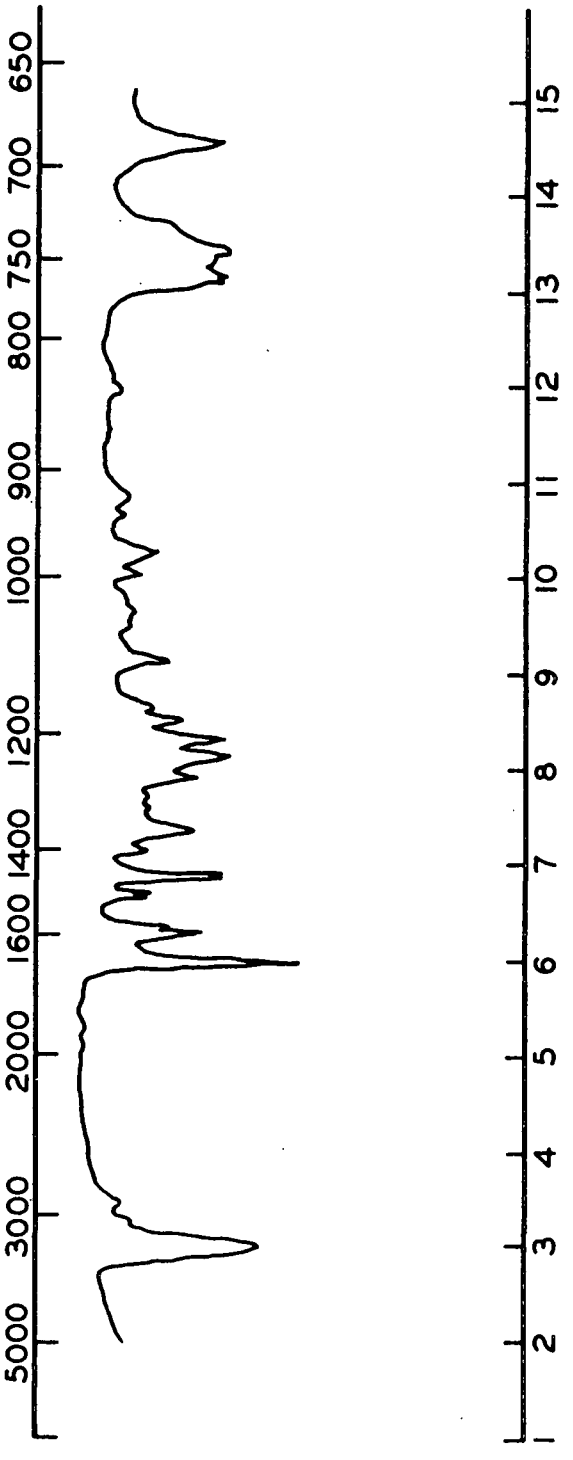


Figure 1. Infrared Spectrum of Compound K

The lack of color in the chalcone solutions indicates that the conjugation and resonance responsible for the color was disrupted, but recovery of the chalcone by extraction indicates that whatever change occurred was very readily reversible. One possible explanation for both the slow chalcone dissolution and the absence of color involves the formation of a very labile carbonyl addition product with bisulfite or some other species in the solution. This would disrupt the conjugation responsible for the chalcone color and probably dissolve the compound. Upon extraction the labile addition product would be destroyed, regenerating the chalcone.

REDUCTION OF FLAVYLIUM SALTS

Solutions of all flavylium salts and pseudo-base preparations from cyanidin chloride were immediately decolorized when sodium hydrosulfite was added. In two instances the order of addition was changed, the flavylium salt (cyanidin chloride) being added to a neutral sodium hydrosulfite solution. Again decolorization occurred immediately.

REDUCTION OF CYANIDIN CHLORIDE

Examination of the orange ethyl acetate extracts from cyanidin chloride reduction mixtures by paper chromatography revealed two colorless reduction products, designated Compounds I and II, and a brown material which gave a streak in all solvents. Extracts from reductions of cyanidin pseudo-base preparations contained the same products. The chromatographic properties of these products are given in Table II.

TABLE II

CHROMATOGRAPHIC BEHAVIOR OF CYANIDIN CHLORIDE REDUCTION PRODUCTS

Component	R_f Values				Color Reactions[c]					
	BAW	6% HOAc	H_2O	AHW	vis.	UV	GG	TSA vis.	TSA UV	2,4-d
I	0.71	0.52	0.48	0.65[a]	C	C	YBr	Bl	Bl	bY
II	0.52	0.20	0.25	0.50[a]	C	C	YBr	P	RO	O
Streak	0.0-0.9[b]	0.0-0.8[b]	0.0-0.9[b]	0.0-0.7[b]	Br	A				

[a] In AHW Compound I gave a blue spot at the indicated R_f value, and Compound II gave a pink spot which corresponded to an authentic sample of cyanidin chloride.

[b] Approximate R_f ranges only.

[c] Legend: A = absorbs; C = colorless; R = red; d = dark; Bl = blue; O = orange; Y = yellow; l = light; Br = brown; P = pink; b = bright

Compound II was tentatively designated as a leucoanthocyanidin because of its color reaction with p-toluenesulfonic acid and its behavior in AHW.

Isolation and Characterization of Compound I

The reduction products were separated and Compound I was isolated by the following procedure. Prior to extraction with ethyl acetate, 454 grams of sodium chloride were added to the cyanidin chloride reduction mixture. The mixture was then filtered. A brown, amorphous solid which gave streaks on paper chromatograms was separated. The filtrate was then continuously extracted with ethyl acetate as usual. The extract was concentrated to a small volume on a rotating evaporator, water was added, and the remaining ethyl acetate was evaporated. A water-insoluble fraction was separated by filtration as a brown, amorphous solid which gave streaks on chromatograms. Chromatograms of the aqueous filtrate showed heavy spots

for Compounds I and II, and still more of the brown, streaking material. The aqueous solution was concentrated on a rotating evaporator to about 2 ml. and placed in a refrigerator. Compound I separated from the solution as faintly orange needles. The separation steps are shown schematically in Fig. 2, and the yields of the different fractions are given in this figure and in Table III.

TABLE III

YIELDS OF FRACTIONS FROM CYANIDIN CHLORIDE REDUCTIONS

Fraction	Yield Based on Solids Content of Ethyl Acetate Extract, %[a]	Yield Based on Weight of Original Cyanidin Chloride, %[b]
Salt Water-Insoluble Fraction		11.0
Ethyl Acetate Extract		81.1
Water-Insoluble Fraction	7.8	6.3
Water-Soluble Fraction	not determined	not determined
Compound I	46.0	37.4
Remaining after Recovery of I	46.2[c]	37.4[c]

[a] The basis was the weight of the extract after drying in a desiccator over anhydrous calcium chloride.
[b] The basis was the airdry weight of the cyanidin chloride.
[c] These figures were obtained by difference.

Chromatograms of the aqueous filtrate after recovery of Compound I showed a very faint spot for Compound I, indicating that recovery was not quantitative. In addition, only a faint spot for Compound II was found, along with a heavy brown streak. This indicated that Compound II apparently underwent a polymerization reaction during the isolation procedure and was the source of the brown, amorphous products.

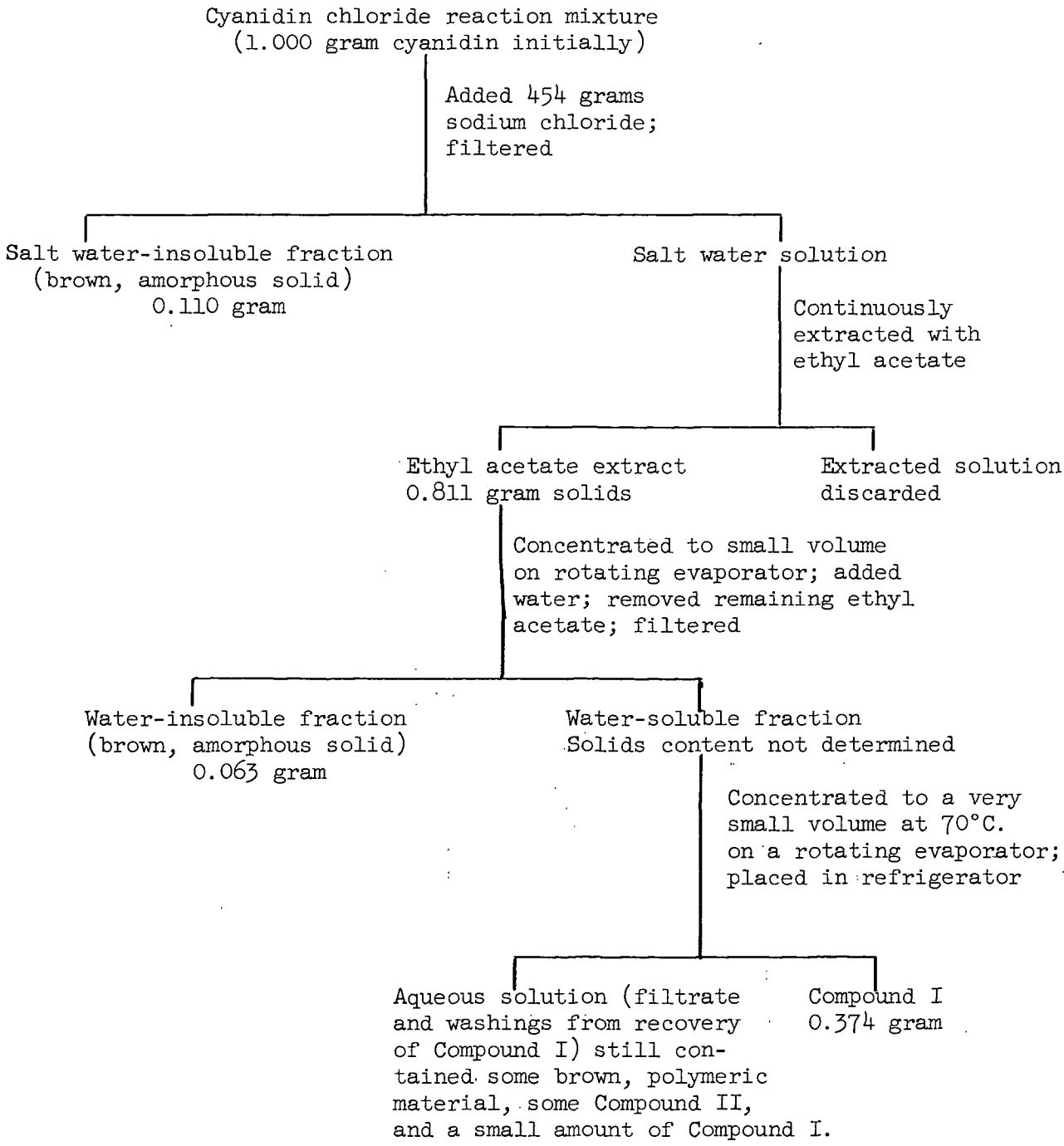


Figure 2. Fractionation of Cyanidin Chloride Reduction Mixtures

Compound I could be recrystallized from water, but from organic solvents alone or paired with water an amorphous form was obtained. Four recrystallizations from water with the aid of activated charcoal gave colorless needles melting at 130 to 131.5°C. with decomposition. Compound I was soluble in hot water, but nearly insoluble in water at room temperature, and was only sparingly soluble in ether. It appeared to be insoluble in 5% aqueous sodium bicarbonate solution and 5% aqueous sodium bisulfite solution. It readily dissolved in 5% aqueous sodium hydroxide solution giving initially a colorless solution which turned dark red within ten minutes. The production of a blue-colored component on chromatograms sprayed with p-toluenesulfonic acid or developed in the AHW solvent was a characteristic reaction of Compound I, but the nature of the blue component was not investigated. The blue color was produced on chromatograms sprayed with n-propanol 0.3N in hydrochloric acid and on chromatograms exposed to hydrochloric acid vapors. It was also produced when an ethanol solution of Compound I was made 0.4N in hydrochloric acid and heated, and it was produced when hydrogen chloride was passed into a glacial acetic acid solution of Compound I.

The crystals of Compound I were dried in vacuo for six hours at 70°C. over anhydrous calcium sulfate, but moisture appeared on the crystal surfaces at 115°C. upon heating in a capillary tube. A sodium fusion test was negative for sulfur and halogens, and no residue was left upon ignition. After four hours of further drying under the above conditions the compound was analyzed for carbon, hydrogen, and oxygen.

Calculated for $C_{15}H_{16}O_7$: C, 58.44%; H, 5.23%; O, 36.33%

Found: C, 58.39%; H, 5.28%; O, 36.03%

The infrared spectrum of Compound I appears in Fig. 3. It shows a strong band at 5.96 microns which indicates carbonyl, a band at 3.40 microns which indicates $-CH_2-$, and various hydroxyl and aromatic bands. Bands characteristic of olefinic unsaturation are absent. The presence of a carbonyl group in Compound I suggested that the above empirical formula might represent a compound containing water of hydration, since the maximum number of hydrogen atoms consistent with a C_{15} carbonyl compound containing two aromatic rings is 14. To determine the empirical formula and to indicate the number of hydroxyl groups, a portion of Compound I was acetylated.

A mixture of 0.16 gram of Compound I, 1 ml. of pyridine, and 1 ml. of acetic anhydride was heated for one hour on a steam bath, kept at room temperature overnight, and then poured into ice water. The resulting tacky yellow solid was recrystallized five times from 95% ethanol to give colorless crystals melting at 91.5 to 93.5°C. After drying this product for two hours in vacuo at 50°C. over anhydrous calcium chloride, the following analytical results were obtained.

Calculated for $C_{25}H_{24}O_{11}$:	C, 59.99%;	H, 4.83%;	O,35.18%;	5 acetyl, 43.00%
Found:	C, 60.01%;	H, 4.81%;	O, 35.18%;	acetyl, 42.9%
	60.09%	4.81%	35.04%	

These values indicated that the empirical formula of the parent hydroxyl compound was $C_{15}H_{14}O_6$, since a compound with this formula containing five hydroxyl groups and a carbonyl group would give a pentaacetate with an empirical formula $C_{25}H_{24}C_{11}$. The infrared spectrum of this acetyl derivative, shown in Fig. 4, contains two bands indicative of carbonyl groups.

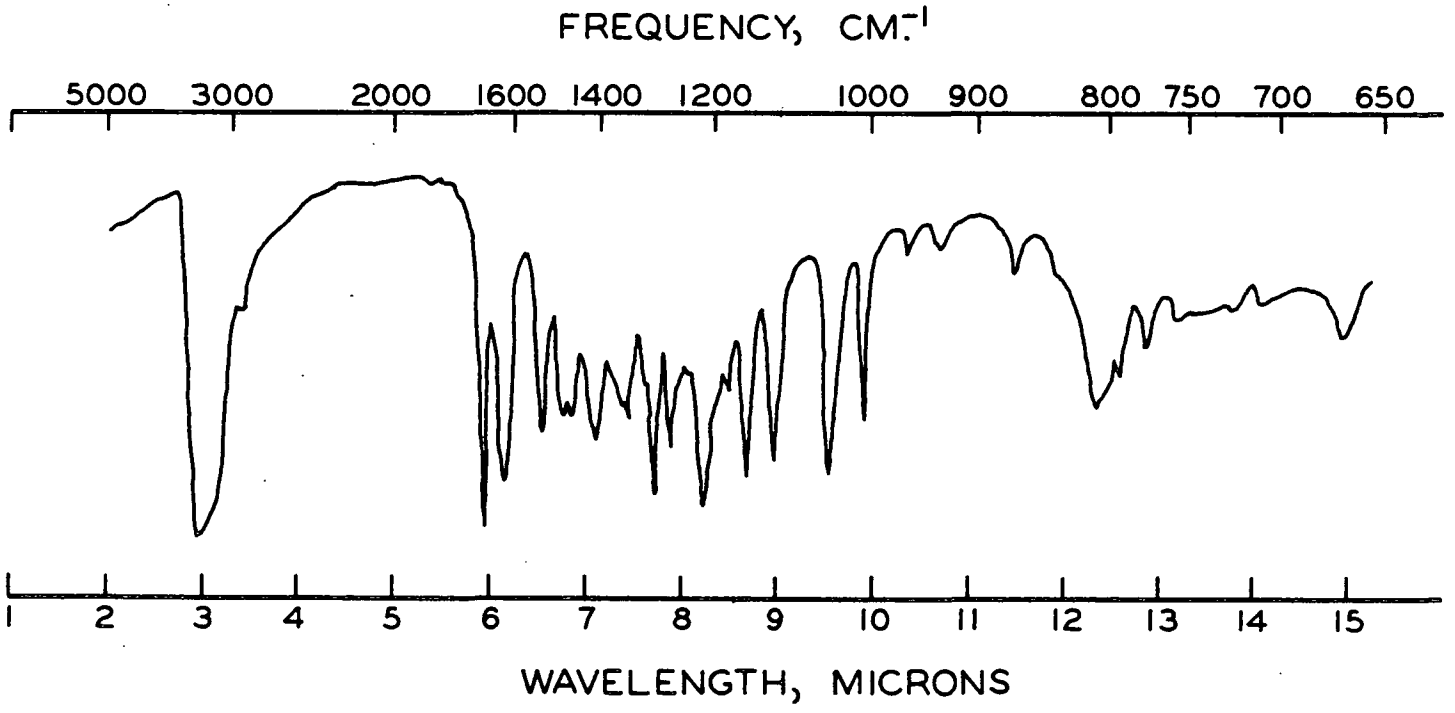


Figure 3. Infrared Spectrum of Compound I

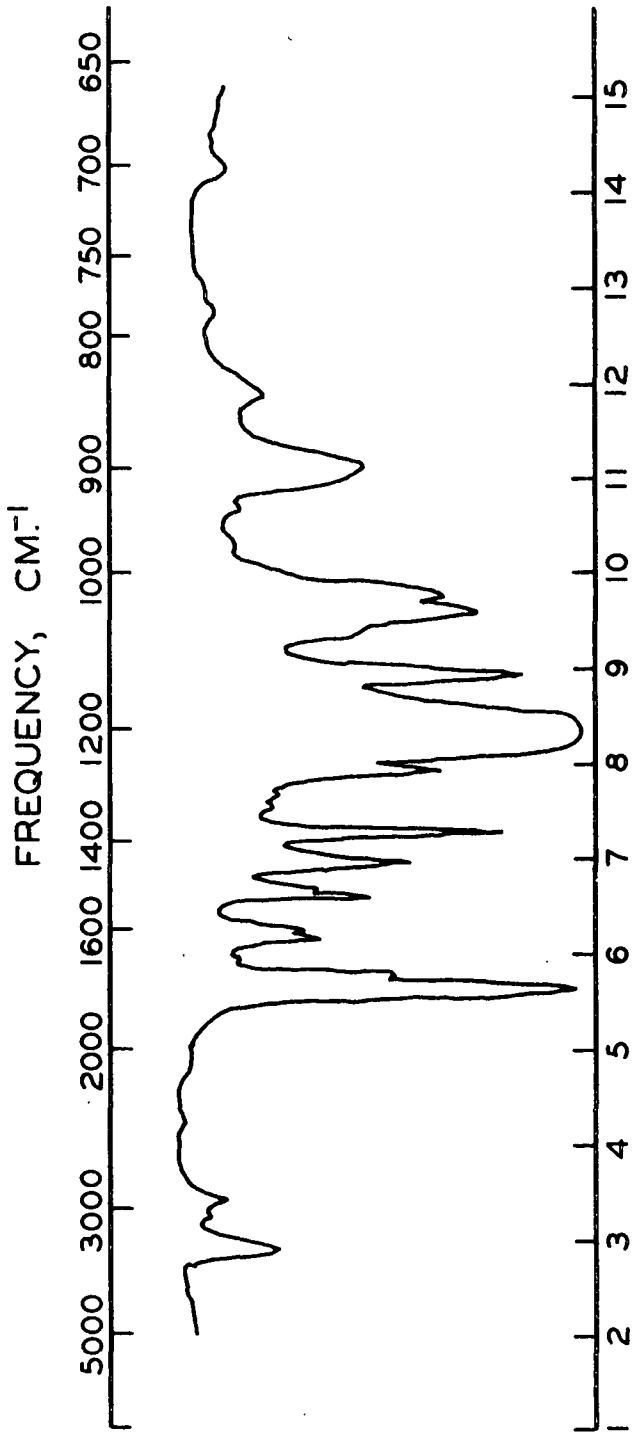
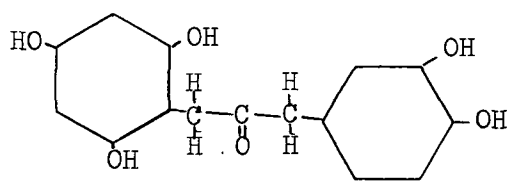


Figure 4. Infrared Spectrum of Pentaacetyl Derivative of Compound I

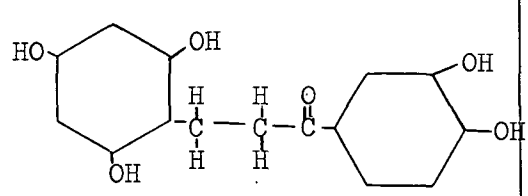
One, at 5.65 microns, is due to the carbonyl in the acetyl groups. The other weak carbonyl band at 5.78 microns is probably due to the original carbonyl group in Compound I. From these data, Compound I, as analyzed, had the empirical formula $C_{15}H_{14}O_6 \cdot H_2O$, and it contained five hydroxyl groups and a carbonyl group.

Hydrosulfite reduction should have produced no change in the phenolic substitution pattern. To verify this, 2 milligrams of Compound I were fused with a pellet of 85% potassium hydroxide. The fusion mixture was diluted with water, acidified, and extracted with ether. The extract was chromatographed along with several simple phenolic compounds having phloroglucinol and pyrocatechol substitution patterns. In this way spots corresponding to phloroglucinol, protocatechuic acid, and pyrocatechol were detected. The location of five of the six oxygen atoms in Compound I was thus determined. These five oxygen atoms must have been present as phenolic hydroxyl groups, since a $C_{15}H_{14}O_6$ carbonyl compound with five oxygens arranged in the indicated pattern cannot contain a heterocyclic ring.

There are three structures which are consistent with the structural features determined thus far. One of these, in which the carbonyl group is located adjacent to the phloroglucinol ring, is eliminated, since it is a known compound melting at 224°C. (88). The two remaining structures are illustrated below.



I



II

Structure I is a substituted dibenzyl ketone. It is the favored structure for Compound I, because cyanidin chloride contains a potential carbonyl group in this position, and because Compound I did not yield a flavylium salt when treated with strong acids, a reported reaction of propiophenones such as Structure II (21,35,36). Neither Structure I nor Structure II is a known compound, but a methoxyl derivative of Structure II is recorded (89). An attempted methylation of Compound I using dimethyl sulfate and potassium hydroxide gave only what appeared to be degradation products. A second attempt using diazomethane gave a brown, amorphous, apparently polymerized material which contained only 28.4% methoxyl and no carbonyl group. A direct comparison of methylated Compound I with the methoxyl derivative of Structure II was thus not achieved.

Kenyon and Mason (90) have reported the reaction of secondary benzyl alcohols with p-toluenesulfinic acid to form sulfones. This reaction has been applied as a specific reaction for benzyl alcohols by Clark-Lewis and Mortimer (91). In the latter work it was found that other secondary alcohols and phenolic hydroxyl groups do not react. If the carbonyl group in Compound I were reduced to a secondary alcohol, a sulfone should be formed by reaction with p-toluenesulfinic acid only if Compound I had possessed the propiophenone Structure II. Compound I (0.1 gram) in methanol was reduced with a solution of 0.003 gram of sodium borohydride in 3 ml. of 0.1N sodium hydroxide. After the reaction had subsided the product was recovered by acidifying the solution with acetic acid, evaporating off the methanol, and extracting with ethyl acetate. Evaporation of the extract gave 0.08 gram of colorless crystals which did not give a carbonyl reaction with 2,4-dinitrophenylhydrazine on

chromatograms. These crystals were treated with sodium p-toluenesulfinate (92) and sulfuric acid under the conditions described by Clark-Lewis and Mortimer (91). When the reaction mixture was poured into ice water a precipitate was obtained. This precipitate gave negative tests for sulfur by a sodium fusion test and by the Feigl method for high oxidation state sulfur (93). A sulfone was, therefore, not produced. This experiment indicated that Compound I does not contain the propiophenone Structure II. Compound I was thus assigned the dibenzyl ketone Structure I.

Isolation and Characterization of Compound II

Because of the extensive polymerization of Compound II which occurred during the isolation of Compound I, Compound II was not isolated from the cyanidin chloride reduction mixtures. Compound II was, however, successfully isolated from a sodium hydrosulfite reduction of the cyanidin pseudo-base. The pseudo-base had been freshly prepared by hydrolyzing cyanidin chloride according to the procedure of Willstätter and Nolan (18). The resulting pinkish orange solution was found, by paper chromatography, to contain a single component. When this solution was treated with sodium hydrosulfite under the conditions of this study, the color was immediately discharged. Chromatograms of the ethyl acetate extract showed a predominant spot for Compound II and a very faint spot for Compound I. By employing the scheme previously used to isolate Compound I, a yield of 0.2 gram of Compound II was obtained.

Compound II crystallized as long, thin, colorless needles. After one recrystallization from water, the crystals melted at 177 to 180°C. with

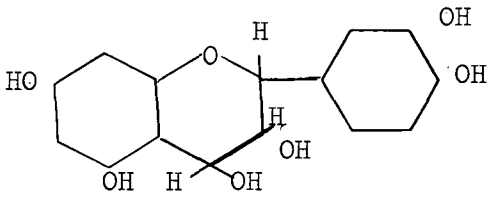
decomposition after darkening at 128°C. Compound II was much more soluble in water than Compound I, and recrystallization from water resulted in a serious loss of product. An amorphous form could be obtained by dissolving the crystals in methanol and pouring the solution into anhydrous ether. Upon standing for one to two days, both the crystals and the amorphous form turned brown, and chromatograms of the brown substance showed only a brown streak.

The red color developed on chromatograms sprayed with p-toluenesulfonic acid indicated that Compound II was a leucoanthocyanidin. An ethanol solution of Compound II was made 0.4N in hydrochloric acid and was heated on a steam bath for 30 minutes. The solution turned red, and chromatograms of the red solution developed in the AHW solvent showed a spot corresponding to authentic cyanidin chloride and a dark brown phlobaphene streak. The presence of cyanidin chloride in the red solution was further indicated when a positive result for cyanidin chloride was obtained upon testing the solution by the Robinsons' procedure for specific flavylium salts (94). The production of a flavylium salt and a phlobaphene material upon acid treatment is the reaction used to denote the leucoanthocyanidins (26,27).

The two types of compounds in the leucoanthocyanidin class, the polymeric leucoanthocyanidins and the flavan-3,4-diols, may be differentiated by their behavior on paper chromatograms. In solvent systems such as BAW the polymeric materials remain near R_f 0.0, whereas the flavan-3,4-diols are mobile (26,27). The R_f value of Compound II, 0.52 in BAW, thus indicates a flavan-3,4-diol. Furthermore, this R_f value is identical

with published values for 3',4',5,7-tetrahydroxyflavan-3,4-diol in BAW (27,30,95). The polymerization of Compound II upon standing is also a property of 3',4',5,7-tetrahydroxyflavan-3,4-diol (30).

The infrared spectrum of Compound II is shown in Fig. 5. This spectrum is consistent with the flavan-3,4-diol structure, and it conforms very closely with spectra for 3',4',5,7-tetrahydroxyflavan-3,4-diol published by Freudenberg and Weinges (95) and by Manson (30). The few minor differences in these spectra are attributed to differences in the spatial configuration of the 2-aryl group and the 3 and 4-hydroxyl groups in the different flavan-3,4-diol preparations.



3',4',5,7-Tetrahydroxyflavan-3,4-diol

The phenolic substitution pattern was verified when phloroglucinol and protocatechuic acid were formed upon fusion of Compound II with potassium hydroxide.

Several attempted preparations of 3',4',5,7-tetrahydroxyflavan-3,4-diol by Manson's procedure (30) gave only brown, amorphous substances which streaked on chromatograms.

Two additional sodium hydrosulfite reductions of fresh cyanidin pseudo-base preparations were made to isolate more of Compound II for further work, but chromatograms of the ethyl acetate extracts from these

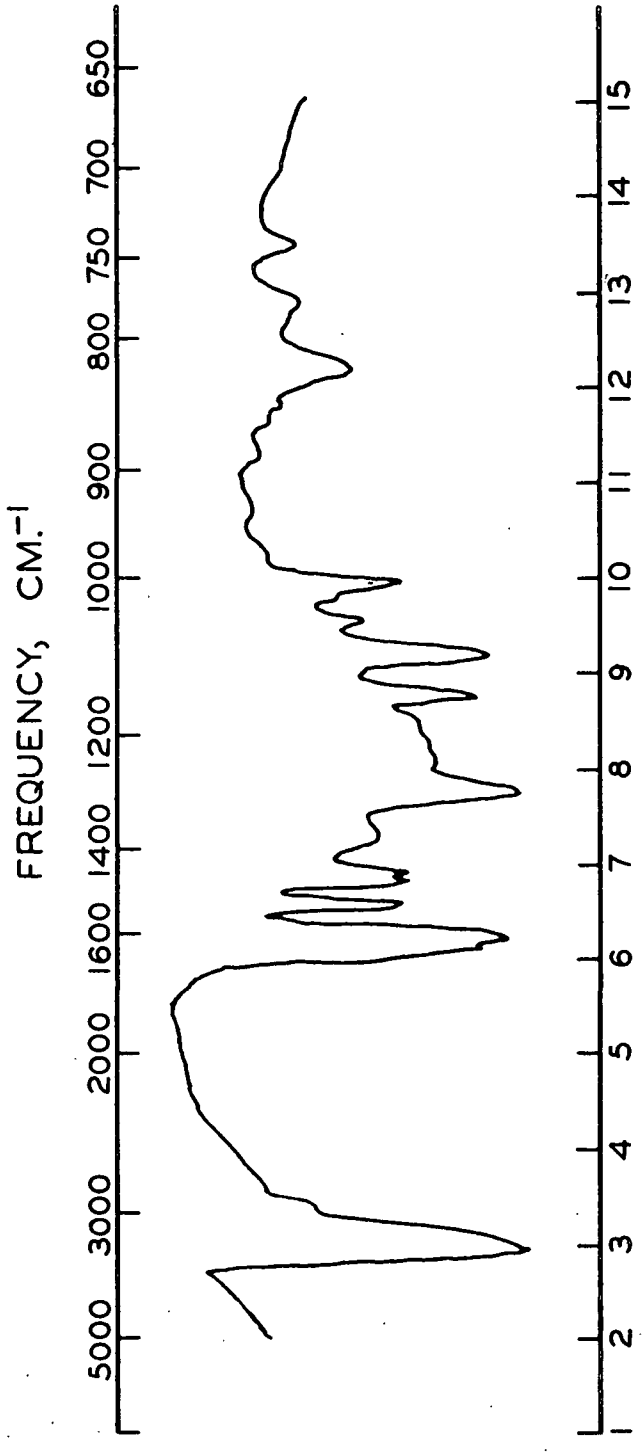


Figure 5. Infrared Spectrum of Compound II

reductions indicated more nearly equal quantities of Compounds I and II than had been the case in the original pseudo-base reduction. The separation scheme now resulted in the crystallization of Compound I, and Compound II underwent the polymerization reaction which had previously prevented its isolation from cyanidin chloride reduction mixtures.

REDUCTION OF OTHER 3-HYDROXYFLAVYLIUM SALTS

Paper chromatograms of ethyl acetate extracts from the reduction of fisetinidin chloride and 3,3',4'-trihydroxyflavylium chloride revealed two colorless reduction products from each of the flavylium salts. On the basis of their chromatographic behavior, these products appeared to be analogous to compounds I and II. The chromatographic properties of these compounds designated III, IV, V, and VI, are given in Table IV.

TABLE IV

CHROMATOGRAPHIC PROPERTIES OF REDUCTION PRODUCTS FROM 3-HYDROXYFLAVYLIUM SALTS

Component	R_f Values				Color Reactions[e]					
	BAW	6% HOAc	H_2O	AHW	vis.	UV	GG	TSA vis.	TSA UV	2,4-d
III[a]	0.79	0.58	0.50	0.77[b]	C	C	BlV	Bl	Bl	bY
IV[a]	0.63	0.32	0.25	0.68[b]	C	C	YBr	P	RO	YO
V[c]	0.82	0.76	0.73	0.89[d]	C	lBl	lV	P	R	O
VI[c]	0.76	0.41	0.46	0.77[d]	C	lBl	lY	P	R	YO

[a] Compounds from fisetinidin chloride.
[b] In AHW Compound III gave a blue spot at the indicated R_f value. Compound IV gave a pink spot which corresponded to a spot for authentic fisetinidin chloride.
[c] Compounds from 3,3',4'-trihydroxyflavylium chloride.
[d] In AHW Compound V gave a pink spot at the indicated R_f value. Compound VI gave a pink spot which corresponded to a spot for authentic 3,3',4'-trihydroxyflavylium chloride.
[e] Legend: Bl = blue; Br = brown; C = colorless; l = light; O = orange; P = pink; R = red; V = violet; Y = yellow; b = bright.

Only Compound III was successfully isolated when the separation scheme used for Compound I was applied to the extracts from the reduction of the above 3-hydroxyflavylium salts. The colorless crystalline product melted at 152 to 155°C. with decomposition after turning bright red at 139°C. The solubility behavior was the same as that reported for Compound I. Fusion with potassium hydroxide yielded resorcinol and protocatechuic acid. The product gave the same blue color reaction with strong acids as Compound I, and the infrared spectrum, shown in Fig. 6, indicates the same type of compound, although the carbonyl band is neither as sharp nor as strong as the band in the spectrum of Compound I. These results indicate that Compound III is analogous to Compound I and is probably the dibenzyl ketone compound.

Compound IV regenerated fisetinidin chloride upon treatment with acids. A complex leucoanthocyanidin which gives fisetinidin chloride is known (96), but the high R_f value of Compound IV, 0.63 in BAW, indicates a flavan-3,4-diol structure. An R_f value of 0.62 in BAW is recorded by Clark-Lewis and Mitsuno (97) for 3',4',7-trihydroxyflavan-3,4-diol.

Compound V, obtained from 3,3',4'-trihydroxyflavylium chloride, gave a pink color reaction with p-toluenesulfonic acid and a pink component was produced when this compound was chromatographed in the AHW solvent. The pink color reaction appeared to be analogous to the blue color reactions of Compounds I and III, however, since the pink component did not correspond to 3,3',4'-trihydroxyflavylium chloride, and the color reactions of the flavylium salts without the 3-hydroxyl group are orange rather than pink. Compound V is probably also a dibenzyl ketone compound. Compound VI

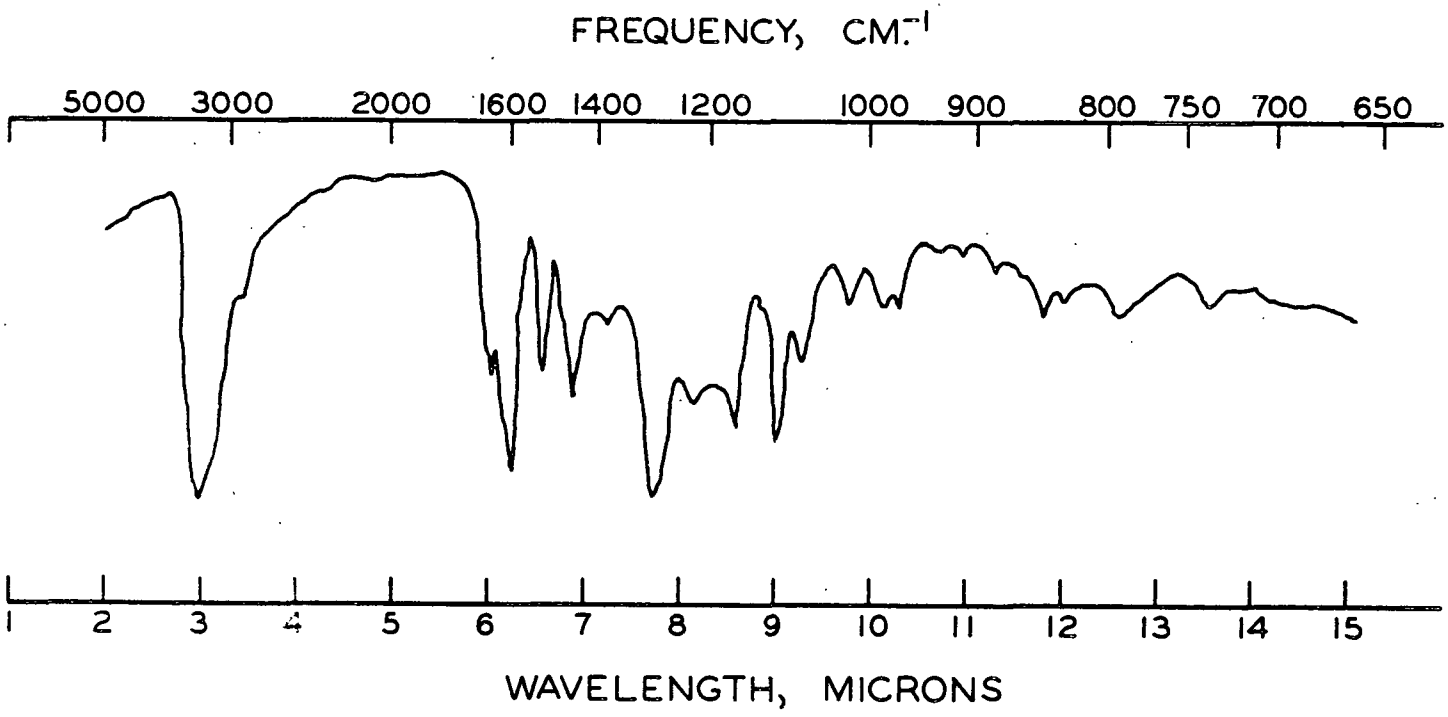


Figure 6. Infrared Spectrum of Compound III

regenerated 3,3',4'-trihydroxyflavylium chloride, and is probably the corresponding flavan-3,4-diol. A complex leucoanthocyanidin which gives this flavylium salt is not known.

REDUCTION OF FLAVYLIUM SALTS WITHOUT 3-HYDROXYL GROUPS

Luteolinidin chloride, butinidin chloride, and 3'-methoxy-4'-hydroxyflavylium chloride gave orange aqueous solutions which were immediately decolorized when sodium hydrosulfite was added. Flavylium chloride, which exists only in a strongly acid solution, yielded a precipitate of 2-hydroxychalcone when it was diluted with water to the volume required for reduction. Chromatographic examination of the ethyl acetate extracts from the reduction of the three stable flavylium salts revealed single colorless products from each flavylium salt. The chromatographic behavior of these products, designated VII, VIII, and IX, is given in Table V.

As indicated in Table V, the reduction products regenerated the flavylium salts from which they were produced upon treatment with strong acids. In this respect, these products are analogous in their behavior to Compounds II, IV, and VI from the 3-hydroxyflavylium salts. The evidence obtained indicates that Compounds II, IV, and VI possess flavan-3,4-diol structures, and by analogy to the behavior of the 3-hydroxyflavylium salts, it would seem that Compounds VII, VIII, and IX should possess flavan-4-ol structures. Flavan-4-ol compounds produce colored components when treated with strong acids, but it is believed that whereas these components are carbonium ions related to the flavylium salts, they are not actually flavylium salts (12). Isolation of Compounds VII, VIII, and IX by the scheme used for Compound I was not successful.

TABLE V

CHROMATOGRAPHIC BEHAVIOR OF REDUCTION PRODUCTS FROM FLAVYLIUM SALTS WITHOUT THE 3-HYDROXYL GROUP

Component	R_f Values						Color Reactions[h]			
	BAW	6% HOAc	H_2O	AHW	vis.	UV	GG	TSA vis.	TSA UV	2,4-d
VII[a]	0.75	--[b]	0.10	0.66[c]	C	C	YBr	O	RO	dY
VIII[d]	0.88	--[b]	0.37	0.75[e]	C	W	YBr	O	O	O
IX[f]	0.94	0.28	0.28	0.89[g]	C	O	V	O	O	dY

[a] Obtained from luteolinidin chloride.
[b] R_f values not measured, because the spots streaked badly.
[c] In AHW an orange spot at the indicated R_f value corresponding to authentic luteolinidin chloride was obtained.
[d] Obtained from butinidin chloride.
[e] In AHW an orange spot at the indicated R_f value corresponding to authentic butinidin chloride was obtained.
[f] Obtained from 3'-methoxy-4'-hydroxyflavylium chloride.
[g] In AHW an orange spot at the indicated R_f value corresponding to authentic 3'-methoxy-4'-hydroxyflavylium chloride was found.
[h] Legend: Br = brown; C = colorless; O = orange; R = red; V = violet; W = white; Y = yellow; d = dark.

The fact that flavylium salts without the 3-hydroxyl group did not give products analogous to Compounds I, III, and V is a further indication that the latter compounds possess dibenzyl ketone structures. If the reaction had produced propiophenone compounds, flavylium salts without the 3-hydroxyl group should have given these compounds also. On the other hand, flavylium salts without the 3-hydroxyl group would not be expected to give dibenzyl ketones, since they contain no enolic hydroxyl group at the 3-position.

MECHANISM OF FLAVYLIUM SALT REDUCTIONS

The experimental evidence obtained indicated that 3-hydroxyflavylium salts reacted with sodium hydrosulfite to produce dibenzyl ketones and

flavan-3,4-diol compounds. A suggested mechanism which accounts for these products is illustrated in Fig. 7, and involves the formation of intermediates of the flavene type. Flavan-3,4-diols would be formed by the addition of water across the double bond of the flavenes, and tautomerism of the 3-hydroxyl group to a ketone followed by reductive cleavage of the benzyl ether linkage would give dibenzyl ketones. The reverse mechanism has been suggested by Bauer and co-workers (32) for the formation of flavylium salts from flavan-3,4-diols upon treatment with acid.

The reaction proceeds very rapidly, as evidenced by the immediate decolorization of the flavylium salt solutions. In an attempt to detect intermediate compounds, a sodium hydrosulfite reduction of cyanidin chloride was stopped soon after the decolorization occurred. It was found that a period of about ten minutes was required from the addition of the sodium hydrosulfite until the reaction mixture could be cooled and extraction with ethyl acetate actually begun. Chromatographic examination of this extract revealed only Compounds I and II. The only difference noted between the composition of the extract from the ten-minute reaction and the hour reaction was the absence of the brown streaking material in the former.

Pseudo-base formation does not appear to be essential as an initial step in the reduction mechanism, even though Compounds I and II were obtained by reducing the cyanidin pseudo-base with sodium hydrosulfite. During the formation of the cyanidin pseudo-base by Willstätter's procedure (18) the deep red cyanidin chloride solution was only slowly lightened in color to a faint but definite pinkish orange. When sodium hydrosulfite was

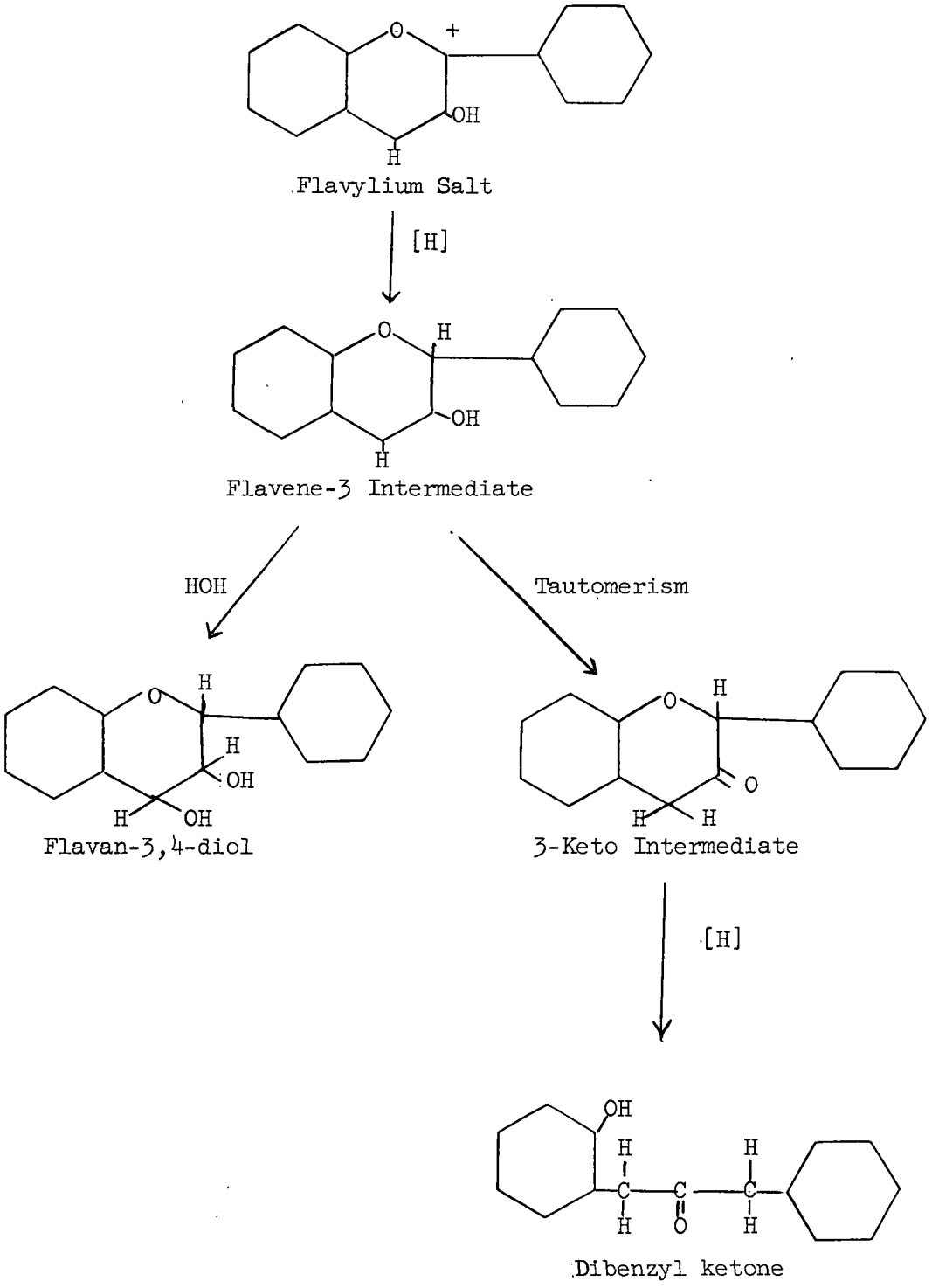


Figure 7. Suggested Mechanism for the Sodium Hydrosulfite Reduction of 3-Hydroxyflavylium Salts

added the solution was immediately decolorized just as cyanidin solutions had been immediately decolorized. These very rapid color changes would appear to indicate an initial reduction step, such as illustrated in Fig. 7, in both cases.

Like the 3-hydroxyflavylium salts, flavylium salts without the 3-hydroxyl group were very rapidly decolorized by sodium hydrosulfite. The reaction mixtures contained single colorless products which regenerated the original flavylium salts, as indicated by chromatographic evidence. On the basis of the behavior of the 3-hydroxyflavylium salts, it would appear reasonable to assign flavan-4-ol structures to the colorless products. However, it is reported (12) that flavan-4-ols react with acid to give colored materials which may not be true flavylium salts. Since the isolation of the reaction products obtained in this work was not successful, it is not possible to assign the flavan-4-ol structure to these products at the present time. More work is indicated to definitely establish whether or not flavan-4-ols yield flavylium salts upon treatment with acid.

SUMMARY AND CONCLUSIONS

The purpose of the present work was to study the effect of sodium hydrosulfite on flavonoid compounds primarily of the colored flavone, chalcone, and flavylium salt classes under conditions approximating those used in bleaching groundwood pulps. Essentially, the flavonoids were treated with dilute aqueous sodium hydrosulfite solutions at pH 7 and 60°C. for one hour in the absence of air. Organic materials were removed by extraction with ethyl acetate, and after a chromatographic examination of the extracts, reduction products, if any, were characterized.

The flavone, quercetin, was insoluble in the reduction medium throughout the course of the reaction, and the unchanged material was recovered quantitatively by filtration. When a soluble form of quercetin, in this case a weak quercetin-magnesium complex, was treated with sodium hydrosulfite at pH 7, some reduction to dihydroquercetin took place. Dihydroquercetin was not reduced by sodium hydrosulfite. Because the solubility characteristics of other flavones are the same as those of quercetin, other flavones were not studied.

The chalcones employed in this study slowly dissolved in the reduction medium to give colorless solutions. However, extraction with ethyl acetate gave intensely yellow-colored extracts from which the unchanged chalcones were quantitatively recovered in all but one case. From the treatment of 1 gram of 2-hydroxychalcone only 6 milligrams of a colorless carbonyl compound which appeared to be the corresponding propiophenone derivative were isolated. The remaining material was unchanged chalcone. The reason

for the lack of color in the hydrosulfite solutions after the chalcones had dissolved is not known, but one possible explanation involves the formation of a very labile carbonyl addition product which, upon extraction, is destroyed to regenerate the chalcone.

Cyanidin chloride, the cyanidin pseudo-base, and other 3-hydroxyflavylium salts reacted very rapidly with sodium hydrosulfite at pH 7 to give two colorless products in each case. On the basis of evidence obtained on the products from the reduction of cyanidin chloride, these compounds appear to be the corresponding dibenzyl ketone derivatives and the corresponding flavan-3,4-diols. Flavylium salts without the 3-hydroxyl group also reacted rapidly with sodium hydrosulfite at pH 7, giving single colorless products in each case. The products appeared to be analogous in their chromatographic behavior to the flavan-3,4-diol compounds from the 3-hydroxyflavylium salts. Although intermediate compounds were not detected, a suggested mechanism which accounts for the products from the 3-hydroxyflavylium salt reductions involves the formation of flavene-3 intermediates. These intermediates could add water across the double bonds to give flavan-3,4-diols, and tautomerism of the flavenes to 3-keto intermediates followed by reductive cleavage of the ether linkages would account for the dibenzyl ketones.

The following conclusions may be drawn from the results of this study:

1. Quercetin is not reduced by aqueous sodium hydrosulfite at pH 7, due primarily to its insolubility in the medium.

2. When rendered soluble at pH 7, quercetin is reduced to the colorless flavanone dihydroquercetin.

3. Dihydroquercetin is not reduced by sodium hydrosulfite at pH 7.

4. The slow dissolution of the chalcones to give colorless solutions in the sodium hydrosulfite reduction medium does not appear to be due to the formation of stable reaction products, since all chalcones except 2-hydroxychalcone were recovered quantitatively, and from 2-hydroxychalcone only 0.6% of a reduction product was found.

5. The evidence indicates that the reduction product from 2-hydroxychalcone is β-[2-hydroxyphenyl]-propiophenone.

6. The 3-hydroxyflavylium salts react rapidly with sodium hydrosulfite at pH 7 to give colorless products which the evidence indicates are the corresponding dibenzyl ketones and the corresponding flavan-3,4-diols.

7. Flavylium salts without the 3-hydroxy group react rapidly to give single colorless products which regenerate the original flavylium salts when treated with strong acids.

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